Utilization of Biomarker Data for Clinical and Environmental Intervention

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Of the 189 air toxics listed in the Clean Air Act, a substantial number are important in potentially causing adverse health effects in several organ systems. Although the major health effects are manifested as respiratory diseases, especially airways disease, these agents may cause cancer and premature mortality, probably from cardiopulmonary disease. Validated biologic markers may be useful in identifying early effects to improve our understanding of exposure–response relationships and clarify susceptibility. However, the knowledge obtained from epidemiologic studies utilizing these new molecular tools will reduce morbidity and mortality from air toxics only when they can be applied effectively in the prevention and control of disease. Intervention strategies using these markers can be used to identify etiologic factors and assess the effectiveness of exposure reduction, and, in some instances, chemoprevention. This paper illustrates examples of these intervention strategies and reviews the current strengths and limitations of environmental molecular epidemiology in controlling disease caused by air toxics. — Environ Health Perspect 104(Suppl 5):921–925 (1996)

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Introduction

This symposium has described the current state-of-the-art research on biologic markers that may be relevant to air toxics. The goal of such research is practical: to apply relevant and valid biomarkers of exposure, effect, and susceptibility to epidemiologic studies of populations exposed to air toxics and to incorporate the resulting information into public health actions aimed at controlling exposures and adverse health effects. Studies that incorporate the use of biologic markers in human populations are often referred to as "molecular epidemiologic studies." A conceptual approach to the application of biologic markers to human populations is shown in Figure 1. The ultimate goal for environmental molecular epidemiology is to fulfill the promise of preventive medicine: environmental disease prevention and control.

Much of what we have learned about the human health effects of toxic exposures has come from epidemiologic studies of exposed workers and from toxicologic studies on animals. Astute clinical observations and animal testing have provided the basis for large-scale epidemiologic studies of workers exposed to toxic agents such as asbestos and vinyl chloride. The risk of diseases caused by such agents are markedly increased in exposed workers who have usually been exposed to concentrations much higher than the general population. However, in the case of community air pollution studies, new challenges have arisen. First, we must find ways to elucidate factors that modestly increase disease risk (*1*). Though such causes could be extremely important from a public health point of view, these kinds of associations are difficult to detect in epidemiologic studies because the relative risk for low-level exposed, versus nonexposed, people is only slightly elevated (*2*). Complicating this problem is the generic aim of exposure–dose assessment in field studies: imprecise methodology to quantify exposures, especially those which may have occurred years ago, can lead to misclassification and bias. Moreover, the temporal pace of the traditional cohort study, which must await data from large populations exposed for long periods, has become an obstacle. Regulators, clinicians, and the public are too impatient to await such "natural experiments," and seek shorter term strategies for studies and quicker solutions (*2*).

Since the concept of molecular epidemiology was popularized in the early 1980s (*3*) to describe an evolving approach to human environmental health research, much work has been done on biomarker development. The theoretical foundation of this work relies on three biologic tenets: *a*) early biologic effects from a toxic exposure are more prevalent and detectable in the population at risk than clinical disease is; *b*) with technological advances, many toxins can be either directly quantified in body fluids or tissues or indirectly measured by identification of some predictable, dose-related (early) biological response; and *c*) either (or both) of the above may be influenced by susceptibility phenomena (heritable or nonheritable), which can also be accounted for with recent advances in molecular biology. Combining these three principles, a new approach to environmental health research has emerged that expands on the traditional epidemiologic paradigm.

In addition to improved measures of exposure, response, and susceptibility, side benefits emerge that have direct relevance for intervention (*4*): contributions to the understanding of pathogenic mechanisms at the tissue, cellular, or molecular

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Abbreviations used: PAH, polycyclic aromatic hydrocarbon; NAT, *N*-acetyltransferase; IL, interleukin.

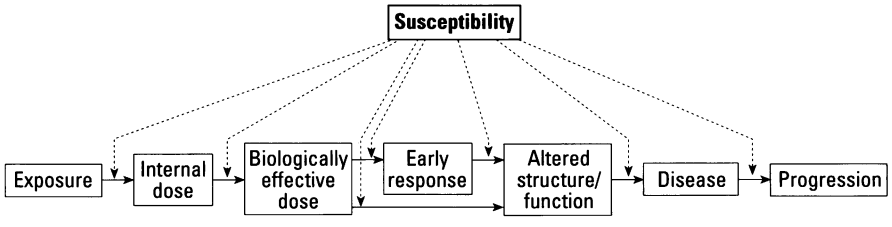

Figure 1. Relationship of biomarkers to exposure, susceptibility, and disease. Adapted from the National Research Council (*36*).

levels, potential for more accurate and etiologic classification of environmental diseases, and the possibility that recognition of early adverse effects could prompt strategies for secondary prevention or disease modification.

Biomarker research does not exist in a vacuum. Its usefulness can only be realized when it translates into prevention strategies to protect public health. In this short review, I describe how biomarkers may be used in clinical and environmental interventions.

Intervention Approaches

Intervention approaches in public health can be characterized as either the high-risk or population approach (*5*). The high-risk approach aims to detect individuals at high risk by using a biologic marker and lowering risk by clinical intervention or other action. Usually, the risk marker is monitored concurrently with the intervention. In contrast, population approach aims to reduce the overall occurrence of a risk factor (exposure) in a population.

Biologic markers may play different roles in each of these approaches. In the high-risk approach, a biologic marker is used to either identify exposure or for early diagnosis of (preclinical) disease. For example, serum cholesterol is an exposure marker of risk indicating biologically effective dose and can be monitored after pharmacologic intervention. Other examples of this approach are shown in Table 1.

An example of the population approach is the prevention of childhood lead accumulation and toxicity by mandating the removal of lead from gasoline. In this approach, biomarkers have played an important role in providing the scientific basis for regulation—an environmental preventive, not clinical, intervention. Biomarkers for lead continued to play an important role in monitoring the intervention itself, demonstrating decrease population lead burden. Other examples of this approach are shown in Table 2.

In some instances, both high-risk and population approaches must be employed simultaneously. Lead toxicity provides an example of this combined approach: regulatory measures are concurrently aimed at reducing the whole population's exposure and at monitoring internal dose (blood lead) of individual children by pediatricians.

These generic intervention strategies have also been important in cancer prevention and control, such as control of occupational bladder cancer by substitution of benzidine dyes with less toxic compounds; control of coke-oven-induced lung cancers by engineering controls and personal protection; and control of bis-chloromethyl ether induced lung cancer by elimination of that compound in plastic and glass production.

Table 1. Examples of high-risk intervention approach.

Problem	Intervention
Coronary heart disease	Lower cholesterol Hypertension control Smoking cessation
Lung cancer	Smoking cessation Radon control in home

Lung Cancer

Tobacco smoke is implicated in up to 85% of the more than 150,000 lung cancer cases that occur in the United States every year. Despite the strong and consistent epidemiologic evidence linking tobacco smoking to cancer, the majority of smokers do not develop lung cancer. Individual variability in carcinogen metabolism may explain differential susceptibility to tobacco-induced lung cancer. In recent years, molecular techniques in the epidemiology of tobacco-related cancer have been applied in three areas: determination of internal and biologically effective dose, detection of early biologic effects, particularly mutations and cytogenetic changes, and assessment of variations in individual susceptibility to carcinogens, mainly via metabolic polymorphisms.

Among the 3800 chemicals that have been identified in tobacco smoke, a large number are biologically active compounds. The most important families of carcinogens are polycyclic aromatic hydrocarbons (PAHs), aromatic amines, nitroso compounds, volatile organic compounds (e.g., benzene, formaldehyde), and radioactive elements such as polonium-210. Chemical compounds derived from tobacco smoke have been measured in biological specimens of smokers and nonsmokers. DNA adducts are formed when chemical carcinogens react with DNA. Several methods of detecting adducts in lung and surrogate tissue, including the sensitive ^{32}P-postlabeling procedure for PAHs, have been described in detail. Among the class of pulmonary carcinogens known as PAHs, benzo[*a*]pyrene forms DNA adducts in the lung that are associated with smoking. Recent research indicates that surrogate tissue (blood mononuclear cells) adducts reflect target organ (lung) adducts in cancer patients (Figure 2) (*6*). Such validation will permit assessment of interventions such as smoking cessation, chemoprevention, and elimination of passive smoking exposures in reducing PAH–DNA adducts in peripheral blood mononuclear cells. Use of blood adducts will also permit epidemiologic study of large populations exposed to PAHs in air pollution.

Table 2. Examples of population intervention approach.

Problem	Intervention
Occupational cancer	Product substitution Engineering controls
Lung cancer	ETS control Radon control
Carbon monoxide poisoning	Auto emission Controls
Lead poisoning	Removal of lead from gasoline and paints

ETS, environmental tobacco smoke.

Although the presence of PAH–DNA adducts has not been definitely associated with lung cancer, it is clear that these adducts appear to be important in the pathway leading to cancer (*7*), and interventions aimed at reducing their presence in high risk individuals are on the horizon. Focusing on this "upstream" marker of dose and early effect is particularly important in lung cancer prevention, a condition for which there is no effective early detection marker and for which treatment is an ineffective means of disease control.

In recent years, mutations in specific genes can be used as "fingerprints" of specific exposures, as surrogate end points of cancer, or for further subtyping of cancer to clarify causal associations (*8*). Epidemiologic evidence suggests the association between chemical exposure,

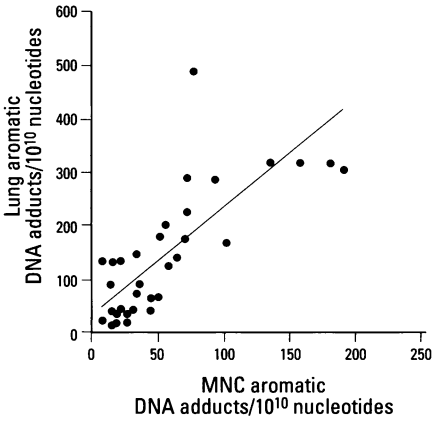

Figure 2. Lung and peripheral blood mononuclear cell (MNC) PAH–DNA adducts. $r = 0.74$; $p < 0.001$.

tumor-suppressor gene (p53) and oncogene (k-*ras*) mutation and cancer occurrence (*9–13*). Investigations concerning the *ras* oncogene family or the p53 tumor-suppressor gene have considered the association between lung cancer and tobacco smoking. Results of these studies suggest that the mutational spectrum of lung cancer depends on whether the person was a smoker and whether the person was exposed to asbestos (*14*).

Genetic susceptibility to lung cancer has been examined in a number of epidemiologic studies that incorporate molecular markers of susceptibility to carcinogens. It has been known for over 30 years that smoking and family history are independent risk factors for lung cancer risk (*15*). Additional evidence has been provided by the study of metabolic polymorphisms, i.e., the different ability to metabolize chemical carcinogens. Traditional monogenic susceptibility to cancer is related to rare diseases (e.g., xeroderma pigmentosa, Li-Fraumeni syndrome) or small subgroups of common diseases (e.g., *BRCA1* in familial breast cancer) and involves rare mutations that can be identified through linkage analysis. Polygenic susceptibility involves frequently occurring genetic polymorphisms, confers a low to medium elevation of risk for frequent diseases, and is identified through epidemiologic (usually case–control) studies. Examples of this include the *CYP2D6* polymorphism phenotype and lung cancer risk (*16*) in whites and the *CYP1A1* and *GSTM1* polymorphisms in lung and bladder cancer, respectively, among Japanese (*17*).

Recent work in the United States suggests that GSTM1 persons with low dietary intake of antioxidants are at high risk of squamous cell carcinoma of the lung, in contrast to persons with null genotype and high antioxidant intake (*18*), after controlling for smoking intensity, duration, age, and time since quitting smoking. This kind of investigation may lead to antioxidant chemoprevention in ex-smokers and illustrates a dual clinical approach to disease control: exposure cessation combined with cumulative risk modification on the secondary level. If genotype-lung cancer risk is modified by a factor amenable to intervention, such as dietary intake of antioxidants (*19*), then dietary changes and/or supplementation may be a useful intervention strategy for individuals who are attempting to quit or have already quit smoking.

Tobacco smoke contains significant amounts of another class of carcinogens, aromatic amines (*20*). Those compounds have been shown to be associated with bladder cancer in smokers (*21*). Epidemiologic studies have suggested that the type of tobacco associated with the highest risk of bladder cancer (air-cured tobacco) is also richer in arylamines and that smokers of air-cured tobacco have higher levels of 4-aminobiphenyl–hemoglobin adducts in their blood compared with smokers of flue-cured tobacco (*22*). Some arylamines (including 4-aminobiphenyl and β-2-naphthylamine) are among the most potent human carcinogens. Measurement of 4-aminobiphenyl–hemoglobin adducts provides evidence of another biologically effective dose marker used in epidemiologic investigation.

Another common polymorphic trait in humans, the *N*-acetylation phenotype, modulates the concentration of 4-aminobiphenyl–hemoglobin adducts in both smokers and nonsmokers (*23*). The *N*-acetyltransferase (NAT) polymorphism has been associated with bladder cancer in epidemiologic studies. NAT is a noninducible enzyme that deactivates aromatic amines (*24*). The slow acetylator genotype prevalence is about 50% in the white population. Slow acetylators exposed to aromatic amines have been reported to have an increased risk of bladder cancer, as well as higher levels of 4-aminobiphenyl adducts (*25,26*).

Nonmalignant Respiratory Disease: Asthma

During the last decade, a number of observations have raised the public health community's interest in asthma: a widespread impression that asthma prevalence has increased in all age groups, dramatic increases in hospital admissions for asthma, an increase in asthma severity despite improved treatments, relatively abrupt changes in the prevalence and nature of asthma in some populations, and an increase in reported asthma mortality from several countries (*27*). Despite difficulties in defining asthma epidemiologically, there is some evidence to suggest that the true prevalence of asthma has been increasing overall, and there is strong evidence to support an increasing prevalence in developing countries and migrant populations. Asthma appears to be more prevalent in Australia, New Zealand, Canada, and England than in the United States and Scandinavia. The role of air pollution (outdoor and indoor) in the etiology, severity, and progression of asthma is a topic of active investigation in many countries today.

Asthma in children is associated with atopy, family history, male gender, parental (especially maternal) smoking, and possibly with acute respiratory illness in childhood. Genetic susceptibility to asthma is best understood by studing its principal underlying co-morbid conditions for which we have phenotype markers: a hypersensitive state or atopy. Atopic individuals suffer from rhinitis, eczema, and the asthma syndrome. Up to 90% of asthmatics between ages 5 and 40 are atopic (*28*).

The central feature of atopic-associated bronchial asthma is the prolonged production of IgE to common inhaled antigens principally triggered by interleukin 4 (IL-4) and T- and B-lymphocyte interaction (*29*). IgE (molecular weight 190 kDa) is unique among the immunoglobulins in having a site at its Fc region with a high affinity for mast cells (which have a high-affinity IgE receptor), but also affinity (via low-affinity receptors) on T- and B-lymphocytes, monocytes and eosinophils (*30*). Allergen-specific IgE, bound to mast cells in the bronchial epithelium, reacts with its target antigen, resulting in activation and degranulation of the mast cell. This results in the release of a number of substances that cause the immediate inflammatory response, including histamine, prostaglandin PGD_2, and leukotrienes, including LTE_4 (slow-reacting substance of anaphylaxis) (*31*). The late-phase bronchial inflammatory response is associated with influx of CD_4 T-lymphocytes and eosinophils (*32*), which may be partly recruited by release of IL-4 from activated mast cells. Activated T-lymphocytes recruit and activate eosinophils by a number of chemokines, especially IL-3 and IL-5 (*33*). The eosinophils release a number of products which further activate mast cells. Eosinophils also release a range of potent mediators, including platelet-activating factor, leukotrienes, and prostaglandins, and activate radicals, which contribute to further inflammatory change (*34*).

Asthma is an inflammatory airway disorder, and the airway response to allergens is mediated through complex mechanisms involving airway inflammation. Current environmental asthma research has focused on how pollutants influence acute airway inflammatory responses in individuals with different host characteristics, including airway responsiveness, IgE antibody isotype, and major histocompatibility complex class II allotypes. We need to understand how host factors modify an individual's acute airways response to air pollutants. For example, host factors characteristic of atopy

may contribute to up-regulation of and modification of the inflammatory response to nonantigenic pollutants (and vice versa).

There are biomarkers of susceptibility and effect available in the study of asthma. Investigation of the genetic susceptibility of atopic asthma has led to direct assay of IgE responsiveness. Measurement of total serum IgE by solid-phase immunoassay offers one method of high-risk phenotype analysis. Altered structure/function can be easily measured using standardized bronchial challenge testing with histamine or methacholine.

Baseline (preexposure) airway hyperresponsiveness may modify the airway response to pollutants, leading to an altered inflammatory response, which in turn may lead to a positive feedback loop whereby the altered inflammatory response further increases airway reactivity. Repeated episodes of pollutant exposure may subsequently lead to airway hyperresponsiveness and clinical asthma. A possible mechanism of this proposed feedback phenomenon is that increased airway responsiveness, as well as other host factors, may modulate the intensity and composition of the cellular infiltrate in the airways after pollutant exposure.

A second possible mechanism of pollutant-associated airway hyperresponsiveness was suggested by Fujimaki et al. (*35*), who showed that, in mice, inhaled particulate pollutants enhance or stimulate IgE antibody production. Fly ash and aluminum silicate instilled to the lung acted as adjuvants for production of IgE and IgG antibody.

Even as our knowledge of the pathogenesis of asthma increases, additional areas of uncertainty continue to arise. The complex interaction between pollutants, allergens, and host characteristics in asthma remain unclear. However, mechanistic research has expanded the range of potential biomarkers available for the study of asthma and air pollution. Borrowing from the paradigm used for cancer, these are obvious parallels in the research approach that can be used to elucidate the causal pathway between exposure and asthma. Clarifying these events may help to guide environmental interventions aimed at minimizing exposure to the whole popultion and guide clinical interventions focused at high-risk individuals.

Conclusions

Current biomarker research in environmental health has fostered the development of a new research endeavor, molecular epidemiology, which expands upon the traditional toxicologic or epidemiologic research paradigms. This development has led to exciting opportunities in environmental health research, which will add greatly to our understanding of the human health effects of toxic exposures. However, much more basic research in environmental molecular epidemiology needs to be accomplished before effective environmental and clinical interventions can be instituted for diseases such as cancer and neurologic disorders. The basis of such interventions will be exposure–disease associations, dose–response relationships, detection of early pathophysiological effects of exposure, and gene–environment interactions. Realization of our goal to prevent disease from air toxics will require expanded multidisciplinary research efforts in both toxicology and epidemiology.

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